



THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

### Mouse models of colorectal cancer as preclinical models

**Citation for published version:**

McIntyre, RE, Buczacki, SJA, Arends, M & Adams, DJ 2015, 'Mouse models of colorectal cancer as preclinical models', *BioEssays*, vol. 37, no. 8. <https://doi.org/10.1002/bies.201500032>

**Digital Object Identifier (DOI):**

[10.1002/bies.201500032](https://doi.org/10.1002/bies.201500032)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

BioEssays

**Publisher Rights Statement:**

© 2015 The Authors. Bioessays published by WILEY Periodicals, Inc.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



# Prospects & Overviews

## Mouse models of colorectal cancer as preclinical models

Rebecca E. McIntyre<sup>1)\*</sup>, Simon J.A. Buczacki<sup>2)</sup>, Mark J. Arends<sup>3)</sup> and David J. Adams<sup>1)</sup>

In this review, we discuss the application of mouse models to the identification and pre-clinical validation of novel therapeutic targets in colorectal cancer, and to the search for early disease biomarkers. Large-scale genomic, transcriptomic and epigenomic profiling of colorectal carcinomas has led to the identification of many candidate genes whose direct contribution to tumourigenesis is yet to be defined; we discuss the utility of cross-species comparative omics-based approaches to this problem. We highlight recent progress in modelling late-stage disease using mice, and discuss ways in which mouse models could better recapitulate the complexity of human cancers to tackle the problem of therapeutic resistance and recurrence after surgical resection.

### Keywords:

colorectal cancer; disease biomarker; mouse model; omics; target identification; target validation

### Introduction

Colorectal cancer (CRC) accounts for 700,000 deaths and 1.4 million newly diagnosed cases globally per annum, making it

the number one cause of non-smoking related cancer deaths [1]. Most CRCs arise in the epithelium, a process driven by genetic and/or epigenetic alterations that result in the formation of premalignant lesions called adenomas (see Table 1 for list of genes most frequently mutated in CRCs). The transition from normal epithelium to benign adenoma is initially driven by alterations that hyperactivate the Wnt pathway (>95% CRCs), and this predominantly occurs through inactivation of the *APC* gene (>80% CRCs) [2–4]. *APC* is a negative regulator of the Wnt pathway and hyperactivation of the Wnt pathway is critical to both the initiation and maintenance of the vast majority of CRCs, although pathway activation is finely tuned [2, 5, 6]. For example, alteration of many genes that are known to regulate Wnt signalling, such as *AXIN2* (Axis inhibition protein 2) and *AMER1* (APC membrane recruitment 1) are found in tumours that also harbour *APC* mutations [2, 7]. The Wnt pathway is also critical for the maintenance of the intestinal epithelium, which undergoes complete renewal every 4–5 days in humans [8]; therefore, it has not been possible to target this pathway in cancers without disrupting intestinal regeneration [9].

Activation of the Ras-MAPK (Kirsten rat sarcoma viral oncogene homolog and mitogen activated protein kinase) pathway, usually via point mutations that constitutively activate *KRAS* or *BRAF* (v-raf murine sarcoma viral oncogene homolog B), and inactivation of the *TGFβ* (transforming growth factor β) pathway through inactivation of *SMAD* gene family members or *TGFβ* receptors, promote the development of advanced adenomas or invasive adenocarcinomas [3]. Over time, a small proportion of advanced adenomas acquire further molecular abnormalities that transform them to invasive and then metastatic carcinomas. Inactivation of *TP53* (Tumour protein p53) or *IGF2R* (Insulin-like growth factor 2 receptor) occurs with greater frequency in established carcinomas that invade submucosal layers than in adenomas [3, 10]. However the molecular alterations that support metastases are poorly understood; at the genomic level at least, it would appear that there is high concordance between primary CRCs and their matched metastatic lesions, which suggests that mechanisms other than gene mutation may be responsible for progression to metastatic disease—for example epigenetic or post-translational modifications [10–12].

DOI 10.1002/bies.201500032

<sup>1)</sup> Experimental Cancer Genetics, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

<sup>2)</sup> Li Ka Shing Centre, Cancer Research UK Cambridge Institute, Cambridge, UK

<sup>3)</sup> Edinburgh Cancer Research UK Centre, University of Edinburgh, Edinburgh, UK

### \*Corresponding author:

Rebecca E. McIntyre  
E-mail: rm5@sanger.ac.uk

### Abbreviations:

**CRC**, colorectal cancer; **EGF**, epidermal growth factor; **GEMM**, genetically engineered mouse models; **HNPCC**, hereditary non-polyposis CRC; **SNPs**, single-nucleotide polymorphisms; **Tfh**, T follicular helper; **TGFβ**, transforming growth factor β.

**Table 1. Selection of some of the most frequently mutated genes in colorectal cancer**

Human/mouse gene symbol	Frequency (%)	Full gene name	Role in development of CRC
<i>APC/Apc</i>	>80	Adenomatous Polyposis Coli	<ul style="list-style-type: none"> <li>Inactivation of APC is the initiating event in the majority of colorectal cancers [100]</li> <li>APC is a negative regulator of the Wnt pathway</li> <li>Hyperactivation of the Wnt pathway initiates development of CRC by stabilising the <math>\beta</math>-catenin transcription factor, increasing expression of transcription factors such as c-Myc and c-Fos, which regulate expression of cell cycle genes</li> </ul>
<i>TP53/Trp53</i>	~65	Tumor protein p53	<ul style="list-style-type: none"> <li>Loss of TP53 is associated with disease progression [101]</li> <li>TP53 regulates expression of genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism</li> </ul>
<i>KRAS/Kras</i>	~45	Kirsten rat sarcoma viral oncogene homolog	<ul style="list-style-type: none"> <li>Mutation of KRAS (&gt;75% of mutations are at codon 12) and BRAF cause activation of the Ras-MAPK pathway and mutations in these genes are mutually exclusive [2]</li> <li>Activation of Ras-Mapk pathway is associated with disease progression and poor prognosis [102]</li> </ul>
<i>BRAF/Braf</i>	~8	v-raf murine sarcoma viral oncogene homolog B	<ul style="list-style-type: none"> <li>Activation of the Ras-MAPK pathway leads to activation of transcription factors such as c-Myc, and c-Fos, which in turn regulate expression of cell cycle genes</li> </ul>
<i>FBXW7/Fbxw7</i>	~20	F-Box And WD Repeat Domain Containing 7	<ul style="list-style-type: none"> <li>E3 ubiquitin ligase that targets cyclin E and c-Myc (cell cycle regulators) for ubiquitin-mediated degradation [103]</li> <li>Low expression of FBXW7 is associated with poor prognosis [104]</li> </ul>
<i>TGFBR2/Tgfr2</i>	~12	Transforming growth factor, beta receptor 2	<ul style="list-style-type: none"> <li>Alterations in TGF<math>\beta</math> pathway e.g. mutation of <i>TGFBR2</i>, <i>SMAD2</i>, <i>SMAD3</i> and <i>SMAD4</i>, are associated with disease progression [93, 105]</li> </ul>
<i>SMAD2/Smad2</i>	~7	SMAD family member 2, 3, 4	<ul style="list-style-type: none"> <li>The SMAD family are intracellular signal transducers that are activated by TGF<math>\beta</math> receptors and act as transcriptional modulators.</li> </ul>
<i>SMAD3/Smad3</i> <i>SMAD4/Smad4</i>	~5 ~12		
<i>PTEN/Pten</i>	~10	Phosphatase and tensin homolog	<ul style="list-style-type: none"> <li>PTEN dephosphorylates phosphoinositide substrates and negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate (PIP3)</li> <li>Negatively regulates AKT/PKB signaling pathway</li> </ul>
<i>CDK8/Cdk8</i>	~12	Cyclin dependent kinase 8	<ul style="list-style-type: none"> <li>CDK8 regulates the cell cycle by acting as a co-activator for transcription of nearly all RNA Polymerase II-dependent genes</li> </ul>

Endoscopic or surgical resection is routinely used for patients with early premalignant adenomas, as well as for the treatment of most early stage carcinomas and selected patients with late stage or advanced metastatic disease, for whom chemotherapy (or radiotherapy/chemoradiotherapy for rectal cancers) is also a key treatment modality [13]. Liver metastases of colorectal carcinomas occur in about 50% of patients, either at the time of diagnosis or at recurrence, and this is a major cause of CRC-related deaths [13]. Long-term survival of CRC patients is correlated with disease stage at diagnosis, and the 5-year survival rate for patients with metastatic CRC is less than 10% [14].

Aside from our lack of understanding of the molecular events that drive metastases, there are several other plausible explanations for our limited success in treating CRC. Firstly, there are currently few biomarkers that are predictive of early disease, the likelihood of favourable treatment response, recurrence or the development of metastatic disease [10]. Secondly, therapeutic resistance, be it acquired or intrinsic, to several licensed colorectal cancer therapies is a major problem [15]. For example, overexpression of the epidermal growth factor (EGF) receptor is associated with increased metastatic potential and poor prognosis in CRC. In these subgroups of cancer patients, monoclonal antibodies to the

EGF receptor e.g. Cetuximab and Panitumumab, block EGF binding and result in tumour regression [16, 17]. Resistance to therapy eventually develops through a variety of mechanisms including point mutations in the EGF receptor that inhibit Cetuximab binding but not EGF, or activating mutations in *KRAS*, an intracellular signalling pathway that converges with the EGF receptor pathway. Finally, the drug discovery pipeline attrition rate for oncology investigational compounds is extremely high. Only 5% of compounds that show promise in pre-clinical studies are eventually approved for clinical use as the majority fail in Phase I/II trials [18]. One of the reasons for this may be the extensive use of CRC cell lines and xenograft models derived from them in pre-clinical anti-cancer drug testing (reviewed by [18, 19]). Cell culture does not model the interaction of primary tumour cells with surrounding cells (the tumour microenvironment or stroma), the process of organ colonisation by metastatic cancer cells (the 'seed and soil' hypothesis), and the recruitment and activation of the adaptive and innate immune systems during tumorigenesis, which are known to exert selective pressures on cancer cells [20–22]. Several cell culture systems have been developed to make allowances for reciprocal cell communication; examples include organoid cultures, the co-culture of tumour epithelial cells and stromal cells or three-dimensional

cultures [23–25]. While these systems provide important insights into disease biology, they are limited in their ability to model the dynamic interplay between tumour and host. In this review, we summarise the strengths and weaknesses of xenograft models derived from CRC cell lines and primary patient derived material, and genetically modified mouse models of colorectal cancer. We discuss several novel applications of these models in cancer gene validation, biomarker discovery and target validation, and discuss their future roles in cross-species comparative ‘omics’ approaches to understanding metastasis and therapeutic resistance.

## Cell line xenografts for reductionist target validation

Xenografting involves the injection of cells or the surgical transplantation of primary tissue into immune-deficient mice such as the nude mouse, which is athymic and does not produce T-lymphocytes, or the severe combined immune-deficient (SCID) mouse, which has altered adaptive immunity but normal innate immunity, to prevent rejection. It was thought that the large number of commercial colorectal cell lines available would represent the inter-tumour heterogeneity of the human disease, but in reality, few cell lines lead to reliable primary tumour growth, and fewer still to naturally metastatic CRC [26]. Success rates appear to be highest when using the colorectal carcinoma cell lines HCT-116 or HT-29, but

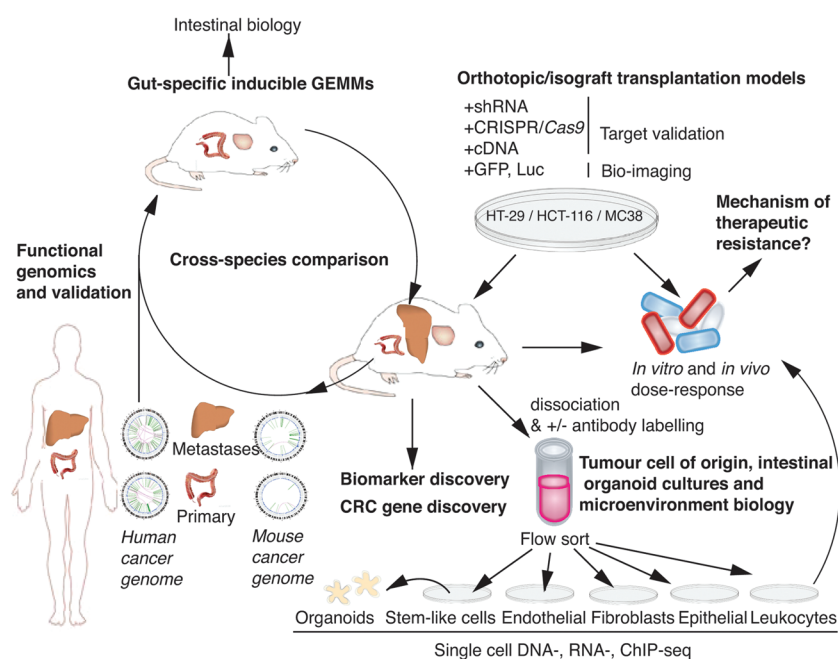
it is not yet clear why this is. Xenograft models are useful for rapid, reductionist, target validation studies, including validation of the many candidate cancer genes identified by The Cancer Genome Atlas (TCGA), however the results must be interpreted with caution (Table 2, Fig. 1 [27, 28]). For example, the environment of subcutaneous tumours is very different from that of autochthonous colorectal carcinomas (i.e. tumours that originate in the location where they are found) and liver metastases; and the extent to which the species mismatch in tumour (human) and stromal (mouse) cells influence tumour growth in vivo is uncertain. CRC stroma is composed of macrophages that secrete matrix-degrading factors such as metalloproteinases, myofibroblasts that secrete Wnt ligands, T-cells that secrete proinflammatory cytokines, and endothelial cells and bone-marrow derived cells, all of which contribute greatly to the growth and progression of this tumour type [20, 22, 29].

## Orthotopic xenografts of CRC cell lines improve tumour-stromal cell cross-talk and the reproducibility of liver metastases

Microenvironmental differences likely explain why the morphology of subcutaneous CRC cell line xenografts, especially the stromal elements, are often markedly different from the human disease and may explain why they have a low

**Table 2.** Transplantation models of colorectal cancer (CRC)

Transplantation model	Strengths	Weaknesses
Subcutaneous CRC cell line xenograft: injection of cells e.g. HCT116 and HT29 to immune deficient mouse	<ul style="list-style-type: none"> <li>• Low cost</li> <li>• Rapid tumour growth (2 weeks)</li> <li>• Well characterized cell-lines: gene mutation status, transcriptome and drug response data available [73, 106, 107]</li> <li>• Easy to genetically manipulate prior to transplantation e.g. with inducible shRNA or by CRISPR/Cas9 [108]</li> <li>• Model accessible to the majority of research labs</li> </ul>	<ul style="list-style-type: none"> <li>• Representative of advanced disease</li> <li>• Have undergone significant clonal selection [73]</li> <li>• Microenvironmental differences (cells derived from colorectum injected under skin)</li> <li>• Species mismatch in tumour (human) and stromal (mouse) cell may limit cross-talk</li> <li>• Immune deficient host</li> <li>• Rarely metastasise</li> </ul>
Orthotopic xenografts of CRC cell lines: injection of cells into intestinal serosa of immune deficient mouse	<ul style="list-style-type: none"> <li>• As above</li> <li>• More natural microenvironment for CRC cells</li> <li>• Some metastasise to liver e.g. from HCT116 or HT29</li> </ul>	<ul style="list-style-type: none"> <li>• As above (except for ‘rarely metastasise’)</li> <li>• May require surgery to implant cells</li> </ul>
Patient-derived xenografts (PDXs): Suturing of 1–2 mm fragments of fresh surgical specimens of CRC, to intestine of immune deficient mouse	<ul style="list-style-type: none"> <li>• Reproducible liver metastasis</li> <li>• Avoid selection of dominant clones during long-term cell culture</li> <li>• Temporary preservation of species-specific tumour-stromal cell cross talk</li> <li>• More natural microenvironment for CRC cells</li> </ul>	<ul style="list-style-type: none"> <li>• Not readily scalable</li> <li>• Host (mouse) stromal cells replace human stromal cells within a few weeks (species mismatch)</li> <li>• Immune deficient host</li> <li>• Limited by availability of surgical specimens</li> <li>• Expensive (labour intensive and time consuming)</li> </ul>
Syngraft/Isograft: Suturing 1–2 mm mouse tumour fragments or mouse tumour cell lines e.g. MC38 cells to a genetically identical inbred, immune competent mouse	<ul style="list-style-type: none"> <li>• No species mismatch between tumour and stromal cells</li> <li>• Host has intact immune system that enables testing of immunomodulatory anti-cancer agents</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive (labour intensive and time consuming)</li> <li>• The model is not human</li> </ul>



**Figure 1.** Mouse models of advanced colorectal cancer (CRC) as research and preclinical tools. The development of genetically engineered mouse models (GEMMs) carrying gene mutations that closely match those found by deep-sequencing of human colorectal carcinomas provide information on the homeostatic and tumorigenic role of a gene in the intestine. Orthotopic transplantation of select colorectal carcinoma cell lines to the caecum of immune-deficient mice results in primary tumour growth and metastases to lymph nodes or liver. Prior to transplantation, colorectal carcinoma cell lines can be genetically manipulated to overexpress (cDNA vector) or inhibit (shRNA or CRISPR-Cas9 vector) a gene of interest to rapidly assess its biological role or to validate potential therapeutic targets. Introducing a reporter gene, such as green fluorescent protein (GFP), to cells prior to transplantation allows in vivo monitoring of primary tumour or metastases growth, which is extremely useful when assessing responses to pharmacological agents or inducible genetic manipulation. Single-cell suspensions from carcinomas can be flow-sorted using cell-specific markers to identify cells of interest, for example stem-like cells for growth of organoids, or to gain further insight into the tumour cells of origin and their interactions with the microenvironment. Cultured cells can be utilised for drug sensitivity screens. Mouse models of advanced CRC can be used to screen for cancer driver genes and disease biomarkers.

metastatic potential [30]. In an attempt to improve the utility of xenograft models, several investigators have systematically tested different variables and found that CRC cell line, site of injection, mouse age and the genetic background of the mouse all markedly affect the frequency of lymph-node or liver metastases [27, 31, 32]. Orthotopic xenografts involve the injection of differentiated cells to a more natural microenvironment i.e. the serosa of the intestine for CRC cell lines, and appears to result in more reproducible liver metastasis [31, 33]. In addition, orthotopic and subcutaneous xenograft models show differing sensitivities to chemotherapeutic agents [30, 31], suggesting that the microenvironment is important for both disease progression and therapeutic

response. Recent refinements to the caecal pouch xenograft method include the non-surgical trans-anal injection of cells into the distal rectum or the microinjection of cell lines to the caecum and engineering cells to express  $\beta$ -human chorionic gonadotropin to support growth [31, 34].

## Patient-derived orthotopic xenografts temporarily preserve tumour-stromal cell cross-talk and improves reproducibility of metastasis

Patient derived xenografts (or 'PDXs') avoid the natural selection of dominant clones and epigenetic and genetic alterations that occur during long-term cell culture as well as temporarily preserving the original, species-specific, tumour-stromal cell interactions. The suturing of small fragments of fresh surgical specimens of CRC liver metastases to the caecum of immune-deficient mice has been reproducible in generating models of metastatic CRC [35, 36]. It was thought that PDXs could be used to test therapies and development of resistance ahead of patient treatment to help personalize cancer therapy, however the approach requires subculture or serial xenografting which currently takes too long [37]. A number of studies have also now reported that host cells replace the human stroma and vasculature of patient derived xenografts, and in the case of CRCs, this appears to occur relatively rapidly (3 weeks vs. 9 weeks for mesothelioma; [38, 39]).

## 'Syngraft' or 'Isograft' models retain species-specific cross-talk between tumour, stroma and immune cells

The two major limitations of all xenograft mouse models of human CRC cell lines are the species mismatch between tumour and stroma, which likely affects cell communication, and the use of immune-deficient hosts. The role of the immune system appears to be particularly important for the development of CRC; T cell immune infiltrate is an important predictive criterion for patient survival [40] and mice with deletion of *Smad4* in T-cells excessively secrete proinflammatory cytokines and develop gastrointestinal tumours [41]. The use of immune-deficient hosts allows development of tumours in the absence of an immune infiltrate, which is critical for CRC tumour progression, and also precludes the testing of immunomodulatory anti-cancer agents. Grafting tumour fragments or the cell lines derived from them, for example MC38 cells (derived from a mouse colon adenocarcinoma), to a genetically identical inbred, immune competent mouse ('syngraft' or 'isograft') is a way of overcoming both of these problems [19].



## Genetically modified mouse models of colorectal cancer retain species-specific cross-talk between tumour, stroma and immune cells

Genetically engineered mouse models (GEMM) offer several advantages over cancer cell lines as research tools in cancer research. Despite their evolutionary distance, the gene content of the mouse and human genomes has been largely conserved through evolution [42], and most cancer pathways are operative in both species [43]. The genomes of inbred laboratory mouse strains are well-characterised and mice offer a 'clean' system in which to test the biological effects of genetic alterations or environmental effects; they are essentially homozygous at every locus and are housed in a controlled environment.

Sequencing of DNA from several tumour types that have developed in mouse or man has recently revealed that human tumours are more heterogeneous than their GEMM-derived counterparts [44, 45]. The difference in complexity of mouse and human tumours is likely due to a combination of factors. Humans have a more varied diet and a different microbiome compared to the laboratory mouse, and so their intestinal cells are exposed to more exogenous genotoxins [46]. In addition, these gene-environment interactions occur over a longer period of time in man; the development of tumours in GEMM is limited by the short lifespan of the mouse or by local research ethics; for example the UK Home Office enforces a tumour size limit of around 1 cm<sup>2</sup>. Furthermore, there is a lack of inter-tumour heterogeneity of GEMM tumours because laboratory mice are inbred and therefore lack the genetic heterogeneity present in the outbred human population. Moreover, GEMM tumours are often initiated and maintained by the same genetic mutation of a strong cancer driver gene, which may limit the number of pathways to tumourigenesis. While the difference in complexity of tumours is advantageous when applying a cross-species oncogenomics approach to cancer gene validation [45], the lack of complexity of mouse tumours poses a problem when trying to faithfully recapitulate the human disease for pre-clinical studies. Some tumour types can be treated in mouse models of cancer, but it is thought that the heterogeneity of human tumours results in a greater potential for resistance to therapy to develop, or for recurrence after surgical resection. Nonetheless, GEMMs of CRC that develop autochthonous intestinal tumours can better model the dynamic interplay between intestinal tumour cells, stroma and the immune system, as well as the response to therapy, when compared with xenograft models.

## Genetically modified mouse models of late stage CRC—25 years in the making

Table 3 summarises some of the genetically modified mouse models (GEMM) of CRC that have been developed over the last 25 years. The first key mouse model of CRC was the multiple intestinal neoplasia (Min) mouse, which arose from a random ethylnitrosourea (ENU) mutagenesis screen [47, 48]. The

*Apc*<sup>Min/+</sup> mouse was subsequently recognised as a paralog for human familial adenomatous polyposis (FAP) syndrome and provided confirmation of the causal gene mutation in sporadic CRCs with 5q21 deletions [49]; later studies showed that somatic inactivation of *APC* is observed in >80% of sporadic CRCs [2].

Hereditary non-polyposis CRC (HNPCC) or Lynch Syndrome, is the most common inherited, autosomal dominant CRC syndrome and accounts for around 3% of all CRCs. HNPCC is most frequently caused by mutations in *MLH1*, *PMS1*, *PMS2*, *MSH2* or *MSH6*, (MutL Homolog 1, Post-Meiotic Segregation Increased 1 and 2 and MutS Homolog 2 and 6, respectively) that encode the enzymatic machinery of DNA mismatch repair. As with sporadic cancers, intestinal cancers from HNPCC patients have a high frequency of mutations in *APC*, as well as *KRAS*, *TP53* and *TGFBR2* [50–52]. Further, approximately 15% of sporadic CRCs display microsatellite instability (MSI) secondary to impaired DNA mismatch repair, frequently because of hypermethylation of the *MLH1* promoter [53]. One of the earliest models that had the potential to recapitulate HNPCC was a homozygous *Msh2*<sup>-/-</sup> deficient mouse [54] (Table 3). Unfortunately, these mice were predisposed to lymphomas, which limited their use as a model of HNPCC. One way to restrict genetic modifications to tissues of interest is to use a *Cre*-recombinase driven by a tissue-specific promoter. *Cre*-recombinases catalyse recombination between two *loxP* sites that are situated in the introns surrounding a critical exon(s) and result in excision of the DNA between the sites. Using a conditional strategy that results in expression of *Cre* recombinase from the *Villin* promoter (intestinal specific gene) a *Villin-Cre/Msh2*<sup>LoxP</sup> mouse was generated that resulted in intestinal adenomas and adenocarcinomas in the absence of lymphoma [55] (Table 3).

The short lifespan of the *Apc*<sup>Min/+</sup> mouse limits the utility of the model and it was thought that mice with fewer tumours would live longer, and that if tumours were restricted to the colon, they might progress to carcinoma and metastasise [56, 57]. A number of different *Apc* mutant mice with germline or inducible cell-type specific conditional alleles of *Apc* were developed (see Table 3 for examples and for an extensive review, see [58]). Despite evidence of malignant transformation and colonic invasion, aged *Apc* mutant mice did not develop metastases, suggesting that to better model the later stages of disease in the mouse, additional genetic mutations would be required (Table 3). *Apc* mutant mice have been crossed to mice with gene mutations found in later stage disease, including *TP53*, *KRAS*, *TGFBR2*, *FBXW7* and *SMAD2-4*, in an attempt to generate more useful pre-clinical models. At best, double mutants displayed more adenocarcinomas, but the mice did not model the metastatic disease (see Table 3).

The many different *Apc* mutant mice that have been created have led to greater understanding of the biology of *Apc*, for example, to ascertain which protein domains and functions of *Apc* are responsible for intestinal tumour initiation and maintenance; it is widely accepted that *Apc*'s role in activation of the Wnt pathway is the major contributor to this process [59]. *Apc* mutant mice have also been used to probe the effect of environmental factors and drugs on intestinal tumourigenesis and stem cell specific *Apc* deletion

**Table 3. Selection of genetically modified mouse models of colorectal cancer**

Mouse allele	Rationale	Strategy	Advantages	Disadvantages
<i>Apc</i> <sup>Min/+</sup> [47, 48]	<ul style="list-style-type: none"> <li>• N/A</li> <li>• Ethylnitrosurea (ENU) mutagenesis screen</li> <li>• Tumour multiplicity easily quantifiable</li> </ul>	<ul style="list-style-type: none"> <li>• Germline truncating mutation (N terminus)</li> <li>• Loss of heterozygosity occurs at wildtype allele in tumours</li> </ul>	<ul style="list-style-type: none"> <li>• Model of human familial adenomatous polyposis (FAP) syndrome and &gt;80% of sporadic CRCs contain mutations [2, 4]</li> <li>• Short-lived for rapid studies</li> </ul>	<ul style="list-style-type: none"> <li>• Developed hundreds of low-grade adenomas</li> <li>• Short-lived as tumour burden causes obstruction, prolapse and bleeding</li> <li>• Short life-span means that adenomas do not acquire sufficient mutations to progress to adenocarcinoma and metastasise [56, 57]</li> <li>• Tumours predominantly located in small rather than large intestine</li> </ul>
<i>Msh2</i> <sup>-/-</sup> [54]	<ul style="list-style-type: none"> <li>• <i>Msh2</i> mutations common in CRC</li> </ul>	<ul style="list-style-type: none"> <li>• Germline knock-out</li> </ul>	<ul style="list-style-type: none"> <li>• Model of Hereditary non-polyposis CRC (HNPCC) or Lynch Syndrome (3% of all CRCs)</li> </ul>	<ul style="list-style-type: none"> <li>• <i>Msh2</i> mutation in all cells of body and mice are predisposed to lymphomas</li> </ul>
<i>Villin-Cre/Msh2</i> <sup>LoxP</sup> [55]	<ul style="list-style-type: none"> <li>• To restrict malignancy to intestine to prevent lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>• Conditional</li> <li>• <i>Cre</i> recombinase<sup>a</sup> expressed from promoter of intestinal specific gene (<i>Villin</i>)</li> </ul>	<ul style="list-style-type: none"> <li>• Developed intestinal adenomas and adenocarcinomas</li> <li>• No deaths from lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop metastases</li> </ul>
<i>Apc</i> <sup>580S/580S</sup> [64]	<ul style="list-style-type: none"> <li>• To model advanced CRC by restricting tumours to colon and reducing tumour burden</li> </ul>	<ul style="list-style-type: none"> <li>• Conditional Adenoviral <i>Cre</i> recombinase<sup>a</sup> is administered through the anus to inactivate <i>Apc</i></li> </ul>	<ul style="list-style-type: none"> <li>• Live &gt;1 year.</li> <li>• Developed two or three intestinal adenomas</li> <li>• Some mice developed adenocarcinomas</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop metastases</li> <li>• Conditional allele (unactivated) reduces <i>Apc</i> expression in all cells, similar to <i>Apc</i><sup>fl/fl</sup> [10], which results in development of life-limiting hepatocellular carcinomas by 14 months (REM unpublished observations of <i>Apc</i><sup>580S/580S</sup>)</li> </ul>
<i>Apc</i> <sup>Lox15/+</sup> , <i>Fabpl-Cre</i> [109]	<ul style="list-style-type: none"> <li>• To model advanced CRC by restricting tumours to the colon and reducing tumour burden</li> </ul>	<ul style="list-style-type: none"> <li>• Conditional</li> <li>• <i>Cre</i>-recombinase<sup>a</sup> is expressed in the distal small intestinal and colonic epithelia to inactivate <i>Apc</i></li> </ul>	<ul style="list-style-type: none"> <li>• Live &gt;1 year.</li> <li>• Developed two or three intestinal adenomas</li> <li>• Some mice developed adenocarcinomas</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop metastases</li> </ul>
<i>Apc</i> <sup>Min/+</sup> <i>Trp53</i> <sup>-/-</sup> [110]	<ul style="list-style-type: none"> <li>• To model advanced CRC through addition of cooperating gene mutations</li> </ul>	<ul style="list-style-type: none"> <li>• Germline mutations in <i>Apc</i> and <i>Trp53</i></li> </ul>		<ul style="list-style-type: none"> <li>• Do not develop metastases</li> </ul>
<i>Apc</i> <sup>2lox14/+</sup> , <i>Kras</i> <sup>LSL-G12D</sup> and <i>Fabpl-Cre</i> [111]	<ul style="list-style-type: none"> <li>• To model advanced CRC through addition of cooperating gene mutations</li> </ul>	<ul style="list-style-type: none"> <li>• Conditional</li> <li>• <i>Cre</i>-recombinase<sup>a</sup> is expressed in the distal small intestine/colonic epithelia and results in constitutive activation of K-Ras and inactivation of <i>Apc</i></li> </ul>	<ul style="list-style-type: none"> <li>• Developed more adenocarcinomas than single mutant (<i>Apc</i> or <i>Kras</i> alone) control mice</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop metastases</li> </ul>
<i>Apc</i> mutant with disruption of <i>Tgfr2</i> , <i>Smad2</i> or <i>Smad4</i> . [112–114]	<ul style="list-style-type: none"> <li>• To model advanced CRC through addition of cooperating gene mutations</li> </ul>		<ul style="list-style-type: none"> <li>• Developed more adenocarcinomas than single mutant control mice.</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop metastases</li> </ul>
<i>Apc</i> <sup>Min/+</sup> <i>Villin-Cre</i> <i>Fbxw7</i> <sup>(ΔG)</sup> [115]	<ul style="list-style-type: none"> <li>• To model advanced CRC through addition of cooperating gene mutations</li> </ul>	<ul style="list-style-type: none"> <li>• Germline mutation of <i>Apc</i></li> <li>• Conditional mutation of <i>Fbxw7</i></li> <li>• <i>Cre</i> recombinase<sup>a</sup> expressed from promoter of intestinal specific gene (<i>Villin</i>)</li> </ul>	<ul style="list-style-type: none"> <li>• Decreased lifespan</li> <li>• Increased tumour burden</li> <li>• <i>Fbxw7</i> null control mice developed adenomas by 9–10 months of age</li> </ul>	<ul style="list-style-type: none"> <li>• Do not develop adenocarcinomas or metastases</li> </ul>
<i>Apc</i> <sup>CKO/CKO</sup> , <i>LSL-G12D</i> ; <i>Kras</i> <sup>tm4tyj/+</sup> [62]	<ul style="list-style-type: none"> <li>• To model advanced CRC through addition of cooperating mutations. To restrict tumours to the colon and reduce tumour burden</li> </ul>	<ul style="list-style-type: none"> <li>• Adenoviral <i>Cre</i> solution administered via the anus to simultaneously disrupt <i>Apc</i> and activate K-ras</li> </ul>	<ul style="list-style-type: none"> <li>• Developed adenocarcinomas after five months</li> <li>• Developed metastases to distant organs after six months e.g. liver</li> <li>• In vivo monitoring via colonoscopy</li> </ul>	<ul style="list-style-type: none"> <li>• Labour intensive mouse surgery required to clamp a section of colon to deliver Adenoviral <i>Cre</i> solution to the colon via the anus</li> </ul>

CRC, Colorectal carcinoma.

<sup>a</sup>*Cre* recombinases catalyse recombination between two *loxP* sites that are situated in the introns surrounding a critical exon(s) and result in excision of the DNA between the sites.

models lent support to the so-called 'bottom-up' model of adenoma formation [60, 61].

One of the most promising models for both the elucidation of the molecular events that support the formation of distant metastases of colorectal cancer and for preclinical investigations is a mouse that has simultaneous, inducible inactivation of *Apc* and activation of *Kras* in the adult colon (Table 3). Six months after surgery 20% of *Apc*<sup>CKO/CKO</sup>-*LSL-Kras* mice (G12D; *Kras*<sup>tm4tyj/+</sup> allele) mice had developed metastases to distant organs such as the liver [62]. It is important to note that expression of wild-type *Apc* may be disrupted by introduction of conditional alleles, which may elevate Wnt signalling levels in tissues other than the intestine, and which appears to drive the formation of life-limiting hepatocellular tumorigenesis before the onset of intestinal tumorigenesis. For example *Apc*<sup>fl/fl</sup> and *Apc*<sup>580S/580S</sup> mice show reduced expression of Apc protein throughout their tissues (in the absence of Cre-mediated recombination of alleles), and develop hepatocellular carcinomas between 9 and 15 months (our unpublished observations of *Apc*<sup>580S/580S</sup> mice; [63, 64]). These findings may preclude the use of some mouse models in studies that aim to understand the events that drive the metastasis of CRC to the liver.

## Mouse models of inflammatory bowel disease and colorectal cancer

Inflammatory bowel diseases (IBD) such as ulcerative colitis predispose patients to developing colorectal cancer as a result of chronic inflammation. It is becoming apparent that these tumours may develop through alternative *TP53* dependent routes compared with classical *APC*-driven tumours [65]. In an attempt to model this disease process mice have been generated that are predisposed to intestinal specific inflammation, including *Il-10*<sup>-/-</sup>, *Il-2*<sup>null</sup> and *Muc2*<sup>-/-</sup> deficient strains [66, 67]. Colitis can also be experimentally induced by the administration of dextran sulfate sodium (DSS) to the drinking water of mice. In addition to mimicking IBD, mice treated this way also produce intestinal tumours [68]. Azoxymethane (AOM) is another commonly used chemical that predisposes to cancer experimentally. AOM is thought to enable base mispairings, and when administered alone or in combination with DSS, AOM reliably produces tumorigenesis in wild type mice [69].

## Genetic screens in mice for validation of cancer 'driver' genes

TCGA has identified genes that are frequently mutated in many different types of malignancy (frequently referred to as 'pan-cancer' genes) suggesting that they are likely to accelerate the process [70]. While these genes are obvious targets for therapy, many are considered intractable drug targets, including MYC and RAS. Within different cancer sub-types there are cancer drivers that could be more

amenable to therapy, however it is difficult to make the distinction between the 'driver' and 'passenger' genes (mutations that drive disease progression and mutations that do not) in patient specimens because of genetic, epigenetic and environmental heterogeneity. In contrast, mouse models, with their homogeneous genetic background and living environment, can be an efficient tool for validating candidate cancer genes (Figs. 1 and 2). Forward genetic screens in mice using insertional mutagenesis have aided the identification or validation of many drivers of cancer, the relevance of which is assessed by comparing the candidates identified through screening with mutations found in the human cancer subtype [45].

One of the first insertional mutagenesis studies to identify novel genetic drivers of intestinal tumorigenesis involved restricting transposition to epithelial cells of the gastrointestinal tract using a *Villin-Cre* recombinase system [71]. Analysis of the common insertion sites for transposon in neoplasms, adenomas and adenocarcinomas revealed that 80% of insertions fell in known CRC genes including *APC*, *FBXW7* (F-box and WD repeat domain containing 7), *PTEN* (phosphatase and tensin homolog), and *SMAD4*, thus validating the approach. The remainder, including *RSPO2* (R-spondin 2), had not been previously implicated in the disease. R Spondin is a ligand for LGR4-6 receptors (LGR, leucine-rich repeat containing G protein-coupled receptor), which stimulate the canonical Wnt signalling pathway. We now know that *RSPO2* fusion genes occur in a subset of colorectal carcinomas and are mutually exclusive with *APC* mutations [7].

To identify mutations that cooperate with inactivation of *Apc* and hyperactivation of the Wnt pathway, two independent teams of researchers mobilised the *Sleeping Beauty* transposon in *Apc*<sup>Min/+</sup> mice or conditional *Apc*<sup>fl/+</sup> mice [6, 72]. The most frequently mutated gene (~85%) in both studies was *Apc*, consistent with the requirement for loss of heterozygosity of *Apc* for intestinal tumour induction. Interestingly, all three mutagenesis screens identified a high number of insertions in the WW domain-containing adaptor with coiled-coil gene (*Wac*), which is also mutated in human cancers [6, 71–73]. *WAC* has recently been shown to be required for activation of the cell-cycle checkpoint in response to DNA damage suggesting that inactivation may cause genomic instability [74]. Both insertional mutagenesis studies of *Apc* mutant mice identified common insertions in the voltage-gated potassium channel subunit *Kcnq1* [6, 72]. Mutations in voltage-gated ion channel subunits have been identified in a variety of human cancers, including colon, and mounting evidence suggests that tumour cells have a depolarised membrane voltage relative to surrounding tissue, which could be exploited for therapy [75]. In support of this finding, *Apc*<sup>Min/+</sup>*Kcnq1*<sup>-/-</sup> double mutant mice developed more intestinal adenomas than *Apc*<sup>Min/+</sup> control mice, and progression to aggressive adenocarcinomas was observed. The authors went on to show that reduced *KCNQ1* expression correlated with poor survival in patients; however, the exact mechanism of tumour suppression remains unknown [76]. These examples prove the utility of transposon-mediated insertional mutagenesis for rapid validation of colorectal cancer genes and for colorectal cancer gene discovery.



## *Apc*<sup>Min/+</sup> mice for early disease biomarker discovery

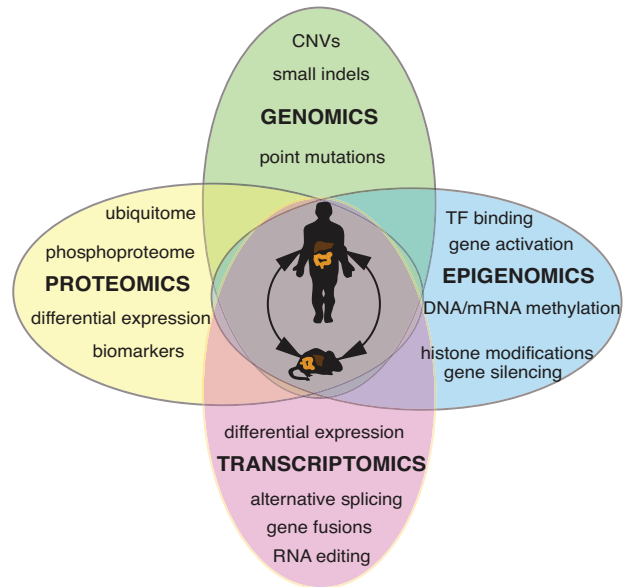
Most screening strategies aimed at the early detection of adenomas and CRC involve endoscopy, and thus have limited applicability. Early detection of CRC is critical for disease management, and so the identification of reliable, early disease biomarkers would improve the outcome for many patients. Mouse models that are predisposed to colorectal tumour formation can be an efficient tool for early disease biomarker discovery (Fig. 2). Since early detection of CRC is critical for disease management, investigators have used mice with germline *Apc* mutations, which model the initiation of intestinal tumorigenesis, to screen for early disease biomarkers. Clusterin was a 'proof-of-principle' for the cross-species approach to biomarker discovery [77]. Upregulation of clusterin was reported in adenomas from *Apc*<sup>Min/+</sup> mice and human tumours, and was recently shown to be highly-expressed in intestinal stem cells [77, 78]. Secreted clusterin is cytoprotective and its prosurvival function forms the basis of the current Phase I/II clinical trials against prostate, lung, and breast cancers [79].

### Cross-species proteomics identifies early disease biomarkers

Recent improvements in proteomic profiling methods, based primarily on mass spectrometry, have allowed the detection and identification of peptides in the blood and biological fluids of cancer patients (Fig. 2). The plasma of tumour-bearing *Apc*<sup>580S/Δ</sup> mice was screened by mass spectrometry, and cathepsin B and D were found to be elevated compared with control mice [80]. Cathepsins are involved in the degradation of the basement membrane and extracellular matrix, a key step in tumour invasion and metastasis. Cathepsin B and cathepsin D are thought to associate with tumour grade of CRC and survival suggesting they may be useful biomarkers of the disease [80, 81]. Similarly, proximal fluid proteome profiling of colon tumours from aged *Apc*<sup>15lox/+</sup> *Fabpl-Cre* mice resulted in the identification of 192 proteins that were more highly excreted by tumours relative to control, including Chitinase 3-like 1 (*Chi3l1*) [82]. CHI3L1 is thought to be involved in processes such as inflammation and tissue remodeling, and was shown to be significantly elevated in the sera of patients with adenomas and advanced adenomas compared with control individuals [83].

### Cross-species epigenomics identifies epigenetic signatures of early disease

Epigenomic profiling of adenomas from *Apc*<sup>Min/+</sup> mice has also been useful in the identification of patient relevant, early disease biomarkers (Fig. 2). Immunoprecipitation of methylated DNA followed by sequencing (known as 'MeDIP-seq') identified regions of the genome were differentially methylated [84]. These regions were mainly enriched for targets of the Polycomb Repressive Complex, the subunits of which, together with those for several DNA methyltransferase



**Figure 2.** Cross-species comparative 'omics' approaches for colorectal cancer (CRC) gene, therapeutic target and early disease biomarker discovery. Despite their evolutionary distance, the gene content of the mouse and human genomes has been largely conserved through evolution and most cancer pathways are operative in both species. Sporadic, low-level mutagenesis of *Apc* and *Kras* in the mouse colon results in a high frequency of invasive adenocarcinomas and liver metastases. Furthermore, serial biopsies of patient tumours is not realistic, but with the development of mouse colonoscopes to guide biopsy collection, this is now a possibility in mouse models. Cancer gene, therapeutic target and biomarker discovery in patient specimens is challenging because of genetic, epigenetic and environmental heterogeneity between patients. Since many of these variables can be controlled for in the inbred laboratory mouse, the comparison of human and mouse primary and metastatic colorectal cancer genomes, transcriptomes, epigenomes, and proteomes including ubiquitinomes, will provide a powerful reductionist tool for identification of the molecular alterations that drive CRC invasion and metastasis. CNV, copy number variation; TF, transcription factor.

complexes, were up-regulated in adenomas. This genome-wide pattern was shown to be partly conserved in human colon carcinomas, suggesting that an epigenetic signature is established early and is retained during progression from adenoma to carcinoma.

### Target identification and validation requires cancer cell lines, xenograft models and GEMM

Not all cancer genes are essential for the sustained growth of established tumours. In addition, tumours may have intrinsic therapeutic resistance, which is believed to arise through natural selection of a latent subclone within a tumour, or acquired therapeutic resistance, which arises through acquisition of a genetic mutation that allows the cancer cell to survive in the presence of the therapeutic agent [15]. It is therefore important to understand both the contribution of a

disease target to tumorigenesis and the mechanisms by which cancers evade targeted therapies. Here, we highlight several recent approaches to the identification and validation of novel therapeutic targets using mouse models of CRC.

Although genetic ablation or overexpression of potential therapeutic targets in CRC cell lines before xenotransplantation does not necessarily mimic pharmacological intervention, nor does it mimic the biology of autochthonous tumours, it may provide a shortcut to target validation (Fig. 1). This approach has recently been useful for the validation of potential therapeutic targets within the Wnt and TGF $\beta$  pathways.

### Targeting the Wnt pathway—reality or drug discovery pipe dream?

Since the Wnt– $\beta$ -catenin signalling pathway is hyperactivated in 93% of colorectal tumours and high Wnt activity defines colon cancer stem cells, the Wnt pathway is the most attractive therapeutic target for colorectal cancer [2, 29, 85]. Despite this, therapeutic agents that specifically target the Wnt pathway have only recently entered clinical trials, and none has yet been approved. The Wnt inhibitor XAV939 inhibits the poly(ADP)-ribosylating (PARP) enzymes tankyrase 1 and tankyrase 2, which interacts with AXIN (acts with APC to destruct  $\beta$ -catenin and dampen Wnt pathway activity) to promote its ubiquitylation and degradation [86]. The development of more selective and potent second-generation tankyrase inhibitors is in progress, however their anti-tumour potential may not be fully realised owing to their intestinal toxicity in pre-clinical mouse models [9]. Rosenbluh and colleagues took a more targeted approach to inhibition of the Wnt pathway: they screened 85 cancer cell lines and found that  $\beta$ -catenin active cancers are dependent on a signalling pathway involving the transcriptional regulator YAP1 (Yes-associated protein 1) [28]. YAP1 and the transcription factor TBX5 were shown to form a complex with  $\beta$ -catenin, and phosphorylation of YAP1 by the tyrosine kinase YES1 lead to localisation of the complex to the promoters of anti-apoptotic genes such as *BIRC5*. To determine whether YAP1 was required for HCT116 xenograft growth they transfected cells with a doxycycline-inducible shRNA to YAP1. Three weeks after injection, tumour growth was restricted to 80% of control animals, suggesting that the YAP1 pathway might be an attractive therapeutic target [28]. Further studies will be required to confirm whether this effect occurs in vivo, where many endogenous ligands that are secreted by stromal cells are known to modify Wnt pathway activity [22].

### Targeting MYC—drugging the undruggable

The transcription factor Myc proto-oncogene protein (Myc) is thought to be a target of the  $\beta$ -catenin–Tcf4 transcription complex in CRC cells in vitro, in normal crypts in vivo and in intestinal epithelial cells acutely transformed on in vivo deletion of the *Apc* gene [59, 87, 88]. Array expression analysis revealed that Myc is required for the majority of  $\beta$ -catenin–Tcf4 transcription complex gene activation following *Apc* loss in mice. While Myc is required for the formation of intestinal

crypts, it is dispensable for homeostasis of the adult intestinal epithelium [89]. Using a combination of conditional mouse models, it was shown that simultaneous inactivation of *Myc* and *Apc* completely ablated tumour development in mice despite high levels of nuclear  $\beta$ -catenin in the intestinal epithelium [90]. Multiple cancer-associated single-nucleotide polymorphisms (SNPs) have been mapped to conserved sequences within a 500-kilobase region upstream of the *MYC* oncogene on human chromosome 8q24, and mice lacking a *Myc* enhancer (*Myc*-335) that includes human SNP rs6983267 are resistant to intestinal tumours induced by the *Apc*<sup>Min</sup> mutation [91]. While these studies have established Myc as a critical mediator of the early stages of intestinal neoplasia following *Apc* loss, it has not yet been possible to pharmacologically regulate Myc. Instead, a selective small-molecule BET bromodomain inhibitor, JQ1, inhibits proteins that are regulatory factors for Myc [92]. Treatment with JQ1 resulted in genome-wide downregulation of Myc-dependent target genes, reduced proliferation and had anti-cancer properties in a mouse model of multiple myeloma, a Myc-dependent hematologic malignancy, suggesting that JQ1 may perform similarly in mouse models of colorectal cancer.

### Pharmacological inhibition of TGFBR1 inhibits metastasis formation

A large proportion of CRCs display mutational inactivation of the TGF $\beta$  pathway. However, paradoxically, they are characterised by elevated TGF $\beta$  production. In other cancer types, such as breast or prostate, which retain a functional TGF $\beta$  signalling pathway, TGF $\beta$  induces a variety of prometastatic programmes that range from induction of epithelial-to-mesenchymal-like transition (or ‘EMT’) to expression of genes that allow colonisation of foreign organs [93]. During 10 years of follow-up after surgical resection of colorectal tumours, only patients with medium or high TGF $\beta$  expression in the primary tumour suffered cancer recurrence, whereas all patients bearing TGF $\beta$ -low stage I, II and III tumours remained disease-free [94]. When Nude mice were inoculated with KM12L4a cells that overexpressed TGF $\beta$ , nearly all developed lung and/or liver metastasis, whereas only one third of control mice developed metastases. It is thought that the metastatic KM12L4a<sup>TGF $\beta$ 1</sup> cells were conferred a survival advantage by suppressing apoptotic stimuli encountered during organ colonization. Moreover, inhibition of TGFBR1 has been shown to inhibit metastasis formation in multiple xenograft models [94, 95]. Since the mechanism is thought to involve cross-talk between tumour and stromal cells, it will be important to show that this regulation occurs in autochthonously occurring liver metastases of CRC, such as those that arise in the conditional *Apc*<sup>CKO/CKO</sup>/*Kras*<sup>G12D</sup> colon cancer model [62].

### Isograft models for validating the role of the immune system in CRC

In a recent study, Bindea and colleagues examined the spatio-temporal dynamics of 28 different immune cell types (the

'immunome') that were infiltrating colorectal tumours were analysed by whole-genome expression, PCR arrays and multicolored immunohistochemistry [96]. The analysis revealed that patients with elevations in genes related to MHC-II, B cell co-stimulation, T cells and Tfh cells experience increased survival. One chemokine in particular, CXCL13, and one cytokine, IL-21, positively correlated with disease-free survival. Bindea and colleagues showed growth acceleration of MC38 isografts in *Cxcr5*<sup>-/-</sup> mice (CXCR5 is the receptor for CXCL13) compared to control, and rejection of tumour cells when recombinant CXCL13 was injected into the colonic submucosa of wild-type mice before transplantation [96]. These data confirmed the prognostic value of CXCL13 in assessing CRC tumour burden, and prove the utility of mouse models in the validation of disease biomarkers.

## Conclusions and outlook

In combination with studies of CRC cell lines and organoid models, mouse models of late-stage CRC will help us to build a comprehensive picture of the molecular alterations that drive tumour formation and disease progression, through cross-species analysis of the genomes, epigenomes, transcriptomes, proteomes and even immunomes of cancers of mice and men. Mouse models will be critical for reductionist approaches that aim to validate the cancer drivers from mutations identified by the TCGA.

Human CRC is the result of interactions between genes, including those involved in host immunity, and the environment, including diet and the microbiome [97, 98]. The mouse affords the ability to investigate the effects of each of these factors independently of each other, which is simply not possible in man. However, recent studies have shown that 'pound for pound', the average mouse tumour is less complex than the average human tumour. These sequencing studies, together with the knowledge that lifestyle factors strongly influence the chances of getting cancer, suggest that the relative simplicity of GEMM tumours is due to the lack of gene-environment interactions in the controlled environment of the laboratory mouse. There is a reasonable body of evidence to suggest that high red meat intake and alcohol consumption are risk factors for CRC, but we are probably only just beginning to understand how our environment affects the course of disease [99]. Modelling the full heterogeneity of human tumours will be required to help tackle the problems of therapeutic resistance and recurrence after surgical resection in CRC; this might be better achieved by generating GEMM that combine a genetic predisposition to CRC with a diet rich in foods associated with increased risk of CRC.

## Acknowledgement

REM, SJAB, MJA and DJA were funded by Cancer Research UK.

## References

1. Torre LA, Bray F, Siegel RL, Ferlay J, et al. 2015. Global cancer statistics, 2012. *CA Cancer J Clin* **65**: 87–108.

2. TCGA. 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**: 330–7.
3. Fearon ER. 2011. Molecular genetics of colorectal cancer. *Annu Rev Pathol* **6**: 479–507.
4. Sjoberg T, Jones S, Wood LD, Parsons DW, et al. 2006. The consensus coding sequences of human breast and colorectal cancers. *Science* **314**: 268–74.
5. Albuquerque C, Breukel C, van der Luijt R, Fidalgo P, et al. 2002. The "just-right" signaling model: APC somatic mutations are selected based on a specific level of activation of the beta-catenin signaling cascade. *Hum Mol Genet* **11**: 1549–60.
6. March HN, Rust AG, Wright NA, ten Hoeve J, et al. 2011. Insertional mutagenesis identifies multiple networks of cooperating genes driving intestinal tumorigenesis. *Nat Genet* **43**: 1202–9.
7. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, et al. 2012. Recurrent R-spondin fusions in colon cancer. *Nature* **488**: 660–4.
8. Van der Flier LG, Clevers H. 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* **71**: 241–60.
9. Lau T, Chan E, Callow M, Waaler J, et al. 2013. A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth. *Cancer Res* **73**: 3132–44.
10. Walther A, Johnstone E, Swanton C, Midgley R, et al. 2009. Genetic prognostic and predictive markers in colorectal cancer. *Nat Rev Cancer* **9**: 489–99.
11. Brannon AR, Vakiani E, Sylvester BE, Scott SN, et al. 2014. Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions. *Genome Biol* **15**: 454.
12. Jones S, Chen W-D, Parmigiani G, Diehl F, et al. 2008. Comparative lesion sequencing provides insights into tumor evolution. *Proc Natl Acad Sci USA* **105**: 4283–8.
13. Van Cutsem E, Nordlinger B, Cervantes A. 2010. Advanced colorectal cancer: ESMO clinical practice guidelines for treatment. *Ann Oncol Off J Eur Soc Med Oncol ESMO* **21**: v93–v97.
14. Jemal A, Bray F, Center MM, Ferlay J, et al. 2011. Global cancer statistics. *CA Cancer J Clin* **61**: 69–90.
15. Garraway LA, Janne PA. 2012. Circumventing cancer drug resistance in the era of personalized medicine. *Cancer Discov* **2**: 214–26.
16. Montagut C, Dalmases A, Bellosillo B, Crespo M, et al. 2012. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* **18**: 221–3.
17. Valtorta E, Misale S, Sartore-Bianchi A, Nagtegaal ID, et al. 2013. KRAS gene amplification in colorectal cancer and impact on response to. *Int J Cancer J Int Cancer* **133**: 1259–65.
18. Sharpless NE, Depinho RA. 2006. The mighty mouse: genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov* **5**: 741–54.
19. Voskoglou-Nomikos T, Pater JL, Seymour L. 2003. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin Cancer Res Off J Am Assoc Cancer Res* **9**: 4227–39.
20. Ernst M, Ramsay RG. 2012. Colorectal cancer mouse models: integrating inflammation and the stroma. *J Gastroenterol Hepatol* **27**: 39–50.
21. Fidler IJ. 2003. The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. *Nat Rev Cancer* **3**: 453–8.
22. Medema JP, Vermeulen L. 2011. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. *Nature* **474**: 318–26.
23. Huh D, Hamilton GA, Ingber DE. 2011. From 3D cell culture to organs-on-chips. *Trends Cell Biol* **21**: 745–54.
24. Jorgensen C, Sherman A, Chen GI, Pasculescu A, et al. 2009. Cell-specific information processing in segregating populations of Eph receptor ephrin-expressing cells. *Science* **326**: 1502–9.
25. Sato T, Vries RG, Snippert HJ, van de Wetering M, et al. 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**: 262–5.
26. Bedard PL, Hansen AR, Ratain MJ, Siu LL. 2013. Tumour heterogeneity in the clinic. *Nature* **501**: 355–64.
27. Flatmark K, Maelandsmo GM, Martinsen M, Rasmussen H, et al. 1990. 2004. Twelve colorectal cancer cell lines exhibit highly variable growth and metastatic capacities in an orthotopic model in nude mice. *Eur J Cancer Oxf Engl* **40**: 1593–8.
28. Rosenbluh J, Nijhawan D, Cox AG, Li X, et al. 2012.  $\beta$ -Catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis. *Cell* **151**: 1457–73.



29. Vermeulen L, De Sousa Melo EF, van der Heijden M, Cameron K, et al. 2010. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* **12**: 468–76.
30. Pocard M, Tsukui H, Salmon RJ, Dutrillaux B, et al. 1996. Efficiency of orthotopic xenograft models for human colon cancers. *Vivo Athens Greece* **10**: 463–9.
31. Hackl C, Man S, Francia G, Milsom C, et al. 2013. Metronomic oral topotecan prolongs survival and reduces liver metastasis in improved preclinical orthotopic and adjuvant therapy colon cancer models. *Gut* **62**: 259–71.
32. Lavilla-Alonso S, Abo-Ramadan U, Halavaara J, Escutenaire S, et al. 2011. Optimized mouse model for the imaging of tumor metastasis upon experimental therapy. *PLoS ONE* **6**: e26810.
33. Jin H, Yang Z, Wang J, Zhang S, et al. 2011. A superficial colon tumor model involving subcutaneous colon translocation and orthotopic transplantation of green fluorescent protein-expressing human colon tumor. *Tumour Biol J Int Soc Oncodevelopmental Biol Med* **32**: 391–7.
34. Cespedes MV, Espina C, Garcia-Cabezas MA, Trias M, et al. 2007. Orthotopic microinjection of human colon cancer cells in nude mice induces tumor foci in all clinically relevant metastatic sites. *Am J Pathol* **170**: 1077–85.
35. Fu XY, Besterman JM, Monosov A, Hoffman RM. 1991. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci USA* **88**: 9345–9.
36. Rashidi B, Gamagami R, Sasson A, Sun FX, et al. 2000. An orthotopic mouse model of remetastasis of human colon cancer liver metastasis. *Clin Cancer Res Off J Am Assoc Cancer Res* **6**: 2556–61.
37. Garralda E, Paz K, Lopez-Casas PP, Jones S, et al. 2014. Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment. *Clin Cancer Res Off J Am Assoc Cancer Res* **20**: 2476–84.
38. Hylander BL, Punt N, Tang H, Hillman J, et al. 2013. Origin of the vasculature supporting growth of primary patient tumor xenografts. *J Transl Med* **11**: 110.
39. Julien S, Merino-Trigo A, Lacroix L, Pocard M, et al. 2012. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* **18**: 5314–28.
40. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, et al. 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**: 1960–4.
41. Kim B-G, Li C, Qiao W, Mamura M, et al. 2006. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. *Nature* **441**: 1015–9.
42. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, et al. 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**: 520–62.
43. Frese KK, Tuveson DA. 2007. Maximizing mouse cancer models. *Nat Rev Cancer* **7**: 645–58.
44. Francis JC, Melchor L, Campbell J, Kendrick H, et al. 2015. Whole-exome DNA sequence analysis of Brca2- and Trp53-deficient mouse mammary gland tumours. *J Pathol* **236**: 186–200.
45. McIntyre RE, van der Weyden L, Adams DJ. 2012. Cancer gene discovery in the mouse. *Curr Opin Genet Dev* **22**: 14–20.
46. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. 2015. How informative is the mouse for human gut microbiota research? *Dis Model Mech* **8**: 1–16.
47. Moser AR, Pitot HC, Dove WF. 1990. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* **247**: 322–4.
48. Su LK, Kinzler KW, Vogelstein B, Preisinger AC, et al. 1992. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* **256**: 668–70.
49. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, et al. 1991. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* **253**: 665–9.
50. Huang J, Papadopoulos N, McKinley AJ, Farrington SM, et al. 1996. APC mutations in colorectal tumors with mismatch repair deficiency. *Proc Natl Acad Sci USA* **93**: 9049–54.
51. Prolla TA, Baker SM, Harris AC, Tsao JL, et al. 1998. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat Genet* **18**: 276–9.
52. Wheeler JM, Bodmer WF, Mortensen NJ. 2000. DNA mismatch repair genes and colorectal cancer. *Gut* **47**: 148–53.
53. Kane MF, Loda M, Gaida GM, Lipman J, et al. 1997. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* **57**: 808–11.
54. Reitnair AH, Schmits R, Ewel A, Bapat B, et al. 1995. MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* **11**: 64–70.
55. Kucherlapati MH, Lee K, Nguyen AA, Clark AB, et al. 2010. An Msh2 conditional knockout mouse for studying intestinal cancer and testing anticancer agents. *Gastroenterology* **138**: 993–1002.
56. Chen X, Halberg RB, Burch RP, Dove WF. 2008. Intestinal adenoma-genesis involves core molecular signatures of the epithelial-mesenchymal transition. *J Mol Histol* **39**: 283–94.
57. Halberg RB, Waggoner J, Rasmussen K, White A, et al. 2009. Long-lived Min mice develop advanced intestinal cancers through a genetically conservative pathway. *Cancer Res* **69**: 5768–75.
58. Zeineldin M, Neufeld KL. 2013. More than two decades of Apc modeling in rodents. *Biochim Biophys Acta* **1836**: 80–89.
59. Sansom OJ, Reed KR, Hayes AJ, Ireland H, et al. 2004. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev* **18**: 1385–90.
60. Barker N, Ridgway RA, van Es JH, van de Wetering M, et al. 2009. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**: 608–11.
61. Roper J, Hung KE. 2012. Priceless GEMMs: genetically engineered mouse models for colorectal cancer drug development. *Trends Pharmacol Sci* **33**: 449–55.
62. Hung KE, Maricevich MA, Richard LG, Chen WY, et al. 2010. Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment. *Proc Natl Acad Sci USA* **107**: 1565–70.
63. Buchert M, Athineos D, Abud HE, Burke ZD, et al. 2010. Genetic dissection of differential signaling threshold requirements for the Wnt/ beta-catenin pathway in vivo. *PLoS Genet* **6**: e1000816.
64. Shibata H, Toyama K, Shioya H, Ito M, et al. 1997. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. *Science* **278**: 120–3.
65. Leedham SJ, Graham TA, Oukrif D, McDonald SAC, et al. 2009. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. *Gastroenterology* **132**: 542–50.
66. Van der Sluis M, De Koning BAE, De Bruijn ACJM, Velcich A, et al. 2006. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* **131**: 117–29.
67. Sohn KJ, Shah SA, Reid S, Choi M, et al. 2001. Molecular genetics of ulcerative colitis-associated colon cancer in the interleukin 2- and beta(2)-microglobulin-deficient mouse. *Cancer Res* **61**: 6912–17.
68. Cooper HS, Murthy S, Kido K, Yoshitake H, et al. 2000. Dysplasia and cancer in the dextran sulfate sodium mouse colitis model. Relevance to colitis-associated neoplasia in the human: a study of histopathology. *Carcinogenesis* **21**: 757–68.
69. Neufert C, Becker C, Neurath MF. 2007. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* **2**: 1998–2004.
70. Kandoth C, McLellan MD, Vandin F, Ye K, et al. 2013. Mutational landscape and significance across 12 major cancer types. *Nature* **502**: 333–9.
71. Starr TK, Allaei R, Silverstein KAT, Staggs RA, et al. 2009. A transposon-based genetic screen in mice identifies genes altered in colorectal cancer. *Science* **323**: 1747–50.
72. Starr TK, Scott PM, Marsh BM, Zhao L, et al. 2011. A Sleeping Beauty transposon-mediated screen identifies murine susceptibility genes for adenomatous polyposis coli (Apc)-dependent intestinal tumorigenesis. *Proc Natl Acad Sci USA* **108**: 5765–70.
73. Forbes SA, Bindal N, Bamford S, Cole C, et al. 2011. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res* **39**: D945–50.
74. Zhang F, Yu X. 2011. WAC, a functional partner of RNF20/40, regulates histone H2B ubiquitination and gene transcription. *Mol Cell* **41**: 384–97.
75. Kale VP, Amin SG, Pandey MK. 2015. Targeting ion channels for cancer therapy by repurposing the approved drugs. *Biochim Biophys Acta* in press. DOI: 10.1016/j.bbmem.2015.03.034
76. Than KLN, Goos JACM, Sarver AL, O'Sullivan MG, et al. 2014. The role of BCNQ1 in mouse and human gastrointestinal cancers. *Oncogene* **33**: 3861–68.
77. Chen X, Halberg RB, Ehrhardt WM, Torrealba J, et al. 2003. Clusterin as a biomarker in murine and human intestinal neoplasia. *Proc Natl Acad Sci USA* **100**: 9530–5.



78. Merlos-Suarez A, Barriga FM, Jung P, Iglesias M, et al. 2011. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* **8**: 511–24.
79. Zoubeydi A, Chi K, Gleave M. 2010. Targeting the cytoprotective chaperone, clusterin, for treatment of advanced cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* **16**: 1088–93.
80. Hung KE, Faca V, Song K, Sarracino DA, et al. 2009. Comprehensive proteome analysis of an Apc mouse model uncovers proteins associated with intestinal tumorigenesis. *Cancer Prev Res Phila Pa* **2**: 224–33.
81. Talieri M, Papadopoulou S, Scorilas A, Xynopoulos D, et al. 2004. Cathepsin B and cathepsin D expression in the progression of colorectal adenoma to carcinoma. *Cancer Lett* **205**: 97–106.
82. Fijneman RJA, de Wit M, Pourghasian M, Piersma SR, et al. 2012. Proximal fluid proteome profiling of mouse colon tumors reveals biomarkers for early diagnosis of human colorectal cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* **18**: 2613–24.
83. Campo E, Munoz J, Miquel R, Palacin A, et al. 1994. Cathepsin B expression in colorectal carcinomas correlates with tumor progression and shortened patient survival. *Am J Pathol* **145**: 301–9.
84. Grimm C, Chavez L, Vilardell M, Farrall AL, et al. 2013. DNA-methylome analysis of mouse intestinal adenoma identifies a tumour-specific signature that is partly conserved in human colon cancer. *PLoS Genet* **9**: e1003250.
85. De Sousa EMF, Vermeulen L, Richel D, Medema JP. 2011. Targeting Wnt signaling in colon cancer stem cells. *Clin Cancer Res Off J Am Assoc Cancer Res* **17**: 647–53.
86. Huang S-MA, Mishina YM, Liu S, Cheung A, et al. 2009. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* **461**: 614–20.
87. He TC, Sparks AB, Rago C, Hermeking H, et al. 1998. Identification of c-MYC as a target of the APC pathway. *Science* **281**: 1509–12.
88. Van de Wetering M, Sancho E, Verweij C, de Lau W, et al. 2002. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* **111**: 241–50.
89. Bettess MD, Dubois N, Murphy MJ, Dubey C, et al. 2005. C-Myc is required for the formation of intestinal crypts but dispensable for homeostasis of the adult intestinal epithelium. *Mol Cell Biol* **25**: 7868–78.
90. Sansom OJ, Meniel VS, Muncan V, Phesse TJ, et al. 2007. Myc deletion rescues Apc deficiency in the small intestine. *Nature* **446**: 676–9.
91. Sur IK, Hallikas O, Vaharautio A, Yan J, et al. 2012. Mice lacking a Myc enhancer that includes human SNP rs6983267 are resistant to intestinal tumors. *Science* **338**: 1360–3.
92. Delmore JE, Issa GC, Lemieux ME, Rahl PB, et al. 2011. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **146**: 904–17.
93. Massague J. 2012. TGFbeta signalling in context. *Nat Rev Mol Cell Biol* **13**: 616–30.
94. Calon A, Espinet E, Palomo-Ponce S, Tauriello DVF, et al. 2012. Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer Cell* **22**: 571–84.
95. Zubeldia IG, Bleau A-M, Redrado M, Serrano D, et al. 2013. Epithelial to mesenchymal transition and cancer stem cell phenotypes leading to liver metastasis are abrogated by the novel TGFbeta1-targeting peptides P17 and P 144. *Exp Cell Res* **319**: 12–22.
96. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, et al. 2013. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* **39**: 782–95.
97. Santarelli RL, Pierre F, Corpet DE. 2008. Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutr Cancer* **60**: 131–44.
98. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. 2012. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. *Nat Rev Microbiol* **10**: 575–82.
99. Crosara Teixeira M, Braghirolli MI, Sabbaga J, Hoff PM. 2014. Primary prevention of colorectal cancer: myth or reality? *World J Gastroenterol WJG* **20**: 15060–9.
100. Kinzler KW, Vogelstein B. 1996. Lessons from hereditary colorectal cancer. *Cell* **87**: 159–70.
101. Hamelin R, Laurent-Puig P, Olschwang S, Jeco N, et al. 1994. Association of p53 mutations with short survival in colorectal cancer. *Gastroenterology* **106**: 42–48.
102. Bos JL. 1989. Ras oncogenes in human cancer: a review. *Cancer Res* **49**: 4682–9.
103. King B, Trimarchi T, Reavie L, Xu L, et al. 2013. The ubiquitin ligase FBXW7 modulates leukemia-initiating cell activity by regulating MYC stability. *Cell* **153**: 1552–66.
104. Iwatsuki M, Mimori K, Ishii H, Yokobori T, et al. 2010. Loss of FBXW7, a cell cycle regulating gene, in colorectal cancer: clinical significance. *Int J Cancer J Int Cancer* **126**: 1828–37.
105. Markowitz S, Wang J, Myeroff L, Parsons R, et al. 1995. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* **268**: 1336–8.
106. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, et al. 2012. Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* **483**: 570–5.
107. Klijn C, Durinck S, Stawiski EW, Haverty PM, et al. 2015. A comprehensive transcriptional portrait of human cancer cell lines. *Nat Biotechnol* **33**: 306–12.
108. Chen S, Sanjana NE, Zheng K, Shalem O, et al. 2015. Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. *Cell* **160**: 1246–60.
109. Robanus-Maandag EC, Koelink PJ, Breukel C, Salvatori DCF, et al. 2010. A new conditional Apc-mutant mouse model for colorectal cancer. *Carcinogenesis* **31**: 946–52.
110. Clarke AR, Cummings MC, Harrison DJ. 1995. Interaction between murine germline mutations in p53 and APC predisposes to pancreatic neoplasia but not to increased intestinal malignancy. *Oncogene* **11**: 1913–20.
111. Haigis KM, Kendall KR, Wang Y, Cheung A, et al. 2008. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet* **40**: 600–8.
112. Hamamoto T, Beppu H, Okada H, Kawabata M, et al. 2002. Compound disruption of smad2 accelerates malignant progression of intestinal tumors in apc knockout mice. *Cancer Res* **62**: 5955–61.
113. Munoz NM, Upton M, Rojas A, Washington MK, et al. 2006. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* **66**: 9837–44.
114. Takaku K, Oshima M, Miyoshi H, Matsui M, et al. 1998. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. *Cell* **92**: 645–56.
115. Babaei-Jadidi R, Li N, Saadeddin A, Spencer-Dene B, et al. 2011. FBXW7 influences murine intestinal homeostasis and cancer, targeting Notch, Jun, and DEK for degradation. *J Exp Med* **208**: 295–312.